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13. ABSTRACT (Maximum 200 Words)
Degradation of the basement membrane (BM) surrounding epithelail units (acini) is associated with tumor progression. It is crucial to understand the molecular mechanisms that underlie the maintenance of an intact BM in order to develop anti-cancer strategies. Using non-neoplastic human breast epithelial S1 cells that differentiate into acini in the presence of extracellular matrix, we have shown a link between the nuclear organization of the protein NuMA, via its C-terminus, and the maintenance of acinar differentiation, notably BM integrity. We have identified a sequence that shares similarities with the histone promoter control 2 (HPC2) protein in the distal portion of the C-terminus of NuMA (NuMA-CTDP). Expression of this sequence in S1 cells prevented acinar differentiation and formation of the BM, and induced a dramatic reorganization of chromatin structure. Fractionation experiments showed that NuMA interacts with the chromatin compartment and suggested that NuMA might be associated with multi-protein complexes. Further sequence analysis of NuMA-CTDP revealed that this region may be restricted to chordates and may adopt a structure possessing organization and signaling properties. Further understanding of the involvement of NuMA in the regulation of chromatin structure is critical to establish whether this protein could become a target for differentiation strategies.

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INTRODUCTION

Differentiation of functional and structural epithelial units of breast tissue, referred to as acini, is maintained by the presence of a continuous basement membrane (BM). The breakdown of the BM by matrix proteases has been associated with loss of structural and functional differentiation and tumor progression. Understanding how an intact BM is maintained in normal epithelial structures should help develop new therapeutic tools to prevent cancer progression. We have reported a link between the organization of the nuclear mitotic apparatus protein NuMA in the cell nucleus, and the activation of matrix proteases and subsequent degradation of the BM (Lelièvre et al., 1998). We have hypothesized that the nuclear organization of NuMA induced by acini is contingent upon NuMA binding to other proteins to form multicomplexes, and that in turn, the supramolecular organization of these multicomplexes is critical for the acinar differentiation stage. In order to decipher the molecular mechanisms that link NuMA organization to the maintenance of acinar differentiation we have proposed to investigate the role of specific sequences of NuMA that we have identified as potential mediators of NuMA functions in the cell nucleus and identify the binding partners of these sequences in conditions where breast epithelial cells are and are not differentiated into acini.

BODY

Our working model is a non-neoplastic human mammary epithelial cell line HMT-3522 (S1) that can be induced to form functional acini surrounded by a complete endogenous BM when cultured in the presence of an exogenous extracellular matrix (Matrigel^{TM}, Becton Dickinson) for 10 days (Petersen et al., 1992). ***Using this system we have investigated the role of NuMA sequences as potential effectors of NuMA functions in the establishment and maintenance of acinar differentiation (statement of work; task 1) and searched for the binding partners of these sequences (statement of work; task 2).***

In the first two reports we showed that there was a putative histone-fold within the C-terminus of NuMA. FLAG-tagged constructs of the histone-fold including or missing the nuclear localization sequence (NLS) located at the N-terminus of this region were expressed in S1 cells. The peptide missing the NLS did not alter the acinar phenotype. We had also shown using affinity binding experiments with purified NuMA-histone fold as a bait that this sequence bound a ≈65 kDa protein in S1 cells but not in S1-derived T4-2 tumor cells. Finally, we had also showed that NuMA was present in the cytoplasm and capable of shuttling between nucleus and cytoplasm (this latter observation will be further investigated as part of a new project).

In this report we show that S1 cells expressing the histone-fold region with the NLS do not differentiate properly, and have altered chromatin organization (task 1 completed). We present new developments regarding the structure and binding capabilities of the histone-fold region, and evidence for NuMA's involvement in chromatin organization and potential participation in multi-protein complexes targeted to chromatin remodeling (task 2, almost completed).

Task 1 (*completed*): Production and analysis of acini expressing truncated forms of NuMA.

Task 1.B of the SOW was the remaining aspect of task 1 to be investigated: *Production of 3D culture of Transfectants and analysis of the phenotype.*
We had identified a sequence with similarities to a histone-fold within the C-terminus of NuMA. Further analysis of the sequence by computational biology with the recent status of the genome and protein database, led us to refine our result and rename this sequence (histone promoter control 2) HPC2-like sequence (residues 1965-2064). Two sequences were stably expressed in S1 cells, a HPC2-like sequence containing the NLS (residues 1965-2101) and a HPC2-like sequence lacking the NLS (residues 2002-2101). We reported last year that expression of the sequence truncated at its N-terminus (hence lacking the NLS) did not affect acinar differentiation. Here we show that selected clones of S1 cells expressing the complete HPC2-like sequence (HPC2-like NuMA-CT S1 cells) do not differentiate properly. 40 and 90% of the acini have an incomplete BM depending on the clone and there is also an increase in apoptosis (figure 1). The distribution of endogenous NuMA is altered compared to cells transfected with vector control and appears as diffuse, similarly to what was observed in non-differentiated cells, instead of the multi-foci distribution typically found in properly differentiated acinar cells (figure 1). Thus, these results confirm data obtained previously by disturbing the binding the C-terminus of NuMA with a C-terminus targeted antibody introduced in live acini (Lelièvre et al., 1998), and indicate that the HPC2-like sequence in NuMA-CT plays a critical role in the establishment of acinar differentiation. Unfortunately these clones do not always maintain the expression of the insert and can only be used for very few passages; they are also difficult to grow following thawing of frozen vials. If we can efficiently revive the frozen batches of clones, we will attempt to measure metalloprotease activity in HPC2-like NuMA-CT S1 acini to assess whether such an activity might be responsible for BM fragmentation. For future easier investigations of NuMA function, we are in the process of implementing the use, in the laboratory, of retroviral constructs with our non-neoplastic cells (they are generally difficult to transfect), in order to increase the efficiency of expression and the rapidity of selection of clones. This will be particularly important for the expression of large peptides of NuMA (e.g., C-terminus truncated) that was unsuccessful so far using classical transfection methods.

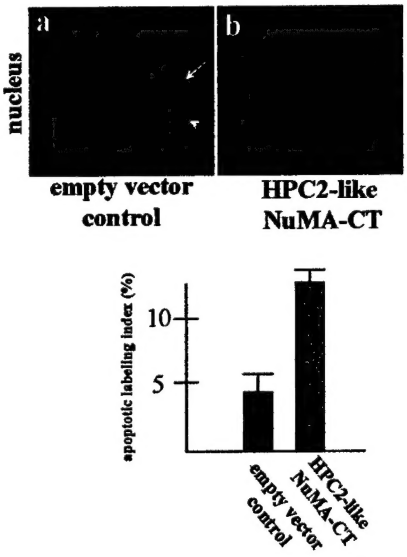

Figure 1. **Expression of HPC2-like NuMA-CT in S1 cells prevents the organization of NuMA into distinct nuclear foci and induces apoptosis**. NuMA immunostaining is shown in red in empty vector control (a, arrows indicates typical ring formations of NuMA foci in the nucleus of the acinar cell), and transfectants (b). The histogram represents the percentage of apoptotic cells, as shown by apoptag staining, in acini formed by cells transfected with the empty vector control and HPC2-like NuMA-CT.

When altering NuMA distribution in acini with antibodies directed against NuMA-CT (Lelièvre et al., 1998) we had observed a redistribution of histone 4 acetylated, suggesting that NuMA organization may have an impact on chromatin. To verify this hypothesis we looked at the distribution of several markers of chromatin structure, including euchromatin marker histone 4 acetylated and heterochromatin markers histone 3 methylated on lysine 9 and histone 4 methylated on lysine

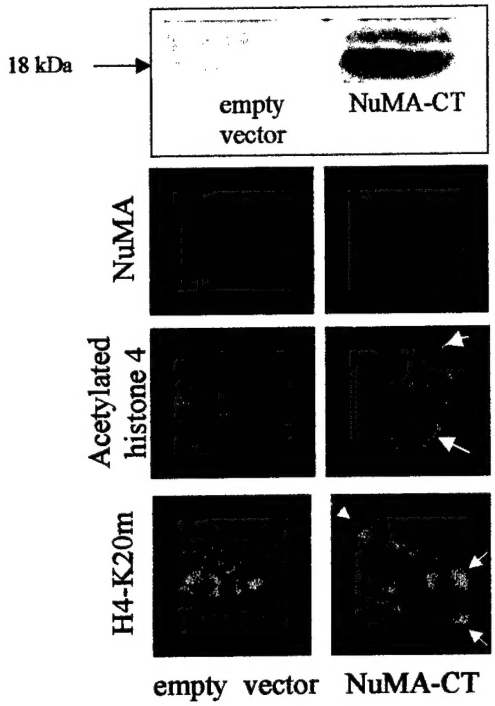

Figure 2. **Alteration of the distribution of endogenous NuMA and chromatin structure in mammary epithelial cell expressing HPC2-like NuMA-CT.** Top panel: Western blot for 18 kDa HPC2-like NuMA peptide in control (empty vector) and FLAG-HPC2-like NuMA-CT transfected S1 cells (NuMA-CT). Other Panels: Immunostaining for NuMA (red), acetylated histone 4 (green) and H4-K20m (green) in control (empty vector) and transfected cells (NuMA-CT). Arrows show the strong H4 acetylated staining all around the nuclear periphery in transfectants. H4K20m foci are mainly located towards the center of the nucleus in control cells, while there are a majority of large foci (arrows) at the nuclear periphery in transfectants. One nucleus is shown per image.

20 in S1 cells transfected with HPC2-like NuMA-CT and empty vector control (figure 2). The distributions of all the chromatin markers investigated so far were altered in HPC2-like NuMA-CT S1 cells compared to controls. These alterations were observed in more than 90% of the nuclei, suggesting that the alteration of chromatin structure may be a direct effect of interfering with endogenous NuMA C-terminus function with the NuMA-CT peptide, rather than the consequence of apoptosis (only 15% of the population undergoes apoptosis). We first investigated whether NuMA was found in the chromatin compartment, since such a location may explain the relationship between alteration in NuMA distribution and change in chromatin structure. Nuclei were isolated from cells cultured under 3-D conditions for 10 days to induce acini formation and treated with micrococcal nuclease to separate the soluble chromatin (digested) and insoluble compartments according to classical chromatin isolation methods. Western blot analysis indicated that NuMA was abundant in the insoluble compartment in agreement with its status of "nuclear matrix" protein, however, it was also highly present in the chromatin compartment (Figure 3). To verify that there had not been any significant cross-contamination between fractions during the preparation, the same nitrocellulose membranes were blotted for chromatin-associated protein MCM3 and nuclear matrix protein lamin B. Each of these proteins was enriched in the compartments that they were supposed to be in, the soluble chromatin compartment for MCM3 and the insoluble compartment for lamin B (Figure 3). We reasoned that if NuMA was found in the chromatin fractions of differentiated cells, then part of the distribution of NuMA observed by immunostaining

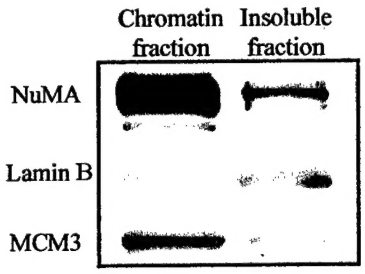

Figure 3. **NuMA is present in the chromatin fraction of non-neoplastic S1 cells.** Chromatin fractionation experiments were performed using classical protocols and up to 5 minutes incubation with 1 unit of micrococcal nuclease. Both the digested chromatin fraction and remaining non-digested fraction (insoluble fraction) are shown. Controls for each fraction include nuclear matrix protein lamin B and chromatin binding protein MCM3.

should be sensitive to DNA degradation. To test this hypothesis, we incubated live cells organized into acini with DNAse1 according to methods previously utilized. Immunostaining of NuMA in control and DNAse1 treated acini revealed that a large portion of NuMA staining was lost upon DNA digestion and NuMA was left mainly all around the nuclear periphery and as a small circle within the cell nucleus (Figure 4). Since DNAse1 only degrades accessible DNA, some DNA usually remains upon treatment with DNAse1. These areas of "non-degraded" DNA are considered to be mainly heterochromatin regions and are commonly located at the nuclear periphery and around the nucleolus of differentiated cells. Dual staining for DNA and NuMA following DNAse1 treatment showed that the remaining NuMA was delineating non-degraded DNA (figure 4). PML, another coiled-coil protein found in nuclear matrix fractions, showed no dramatic alteration in its distribution upon DNAse1 treatment and no particular relationship with the remaining DNA (Figure 4). These data suggest that a fraction of NuMA is sensitive to chromatin removal, while another fraction, non extractable, delineates non-digested DNA.

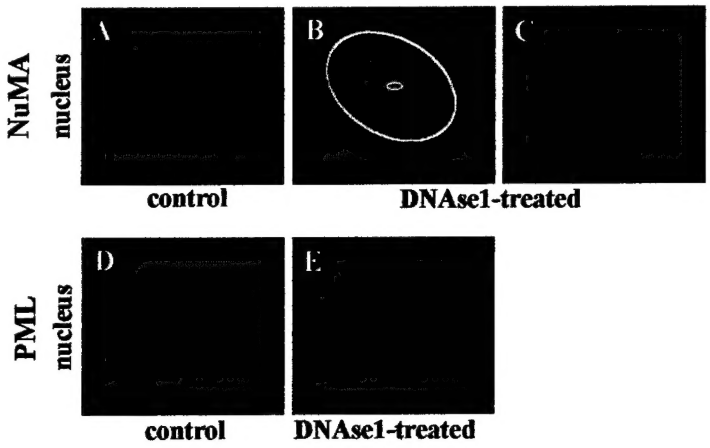

Figure 4. **Part of NuMA is removed upon DNAse 1 treatment**. Acini were treated with DNAse 1 for 40 minutes and immunostained for NuMA (red) and PML (green). NuMA is shown in control (A), and following DNAse1 treatment (B and C); PML is shown in control (D) and following DNAse1 treatment (E). Drawing (in purple) indicates the position of the remaining DNA in image B. DAPI DNA counterstain is shown in blue (C, D and E). Images were recorded using confocal microscopy. One nucleus is shown per image.

As shown in figure 1, S1 cells expressing HPC2-like NuMA-CT have an altered distribution of endogenous NuMA, therefore we checked whether endogenous NuMA has an altered relationship with chromatin in these cells, i.e., we assessed whether it was still affected by chromatin removal. Although the slow growth of our full length HPC2-like NuMA CT transfectants did not allowed us to prepare large cultures that are necessary for chromatin fractionation, we could culture cells in small amounts for *in situ* treatment with DNAse1 and microscopy analysis. S1 transfectants were cultured under 3-D conditions in 4-well chamber slides for 10 days and submitted to DNAse1 digestion. Immunostaining for endogenous NuMA revealed that, in contrast to cells transfected with vector control, NuMA distribution was not affected by DNAse1 treatment in HPC2-like NuMA-CT S1 cells, indicating that the interaction between endogenous NuMA and chromatin had been altered in these cells (Figure 5A). S1-derived T4-2 HMT-3522 cells form tissue-like disorganized tumor nodules with maintenance of cell proliferation and lack of tissue differentiation when cultured in the presence of Matrigel (Weaver et al, 1997). We have observed that malignant T4-2 cells show a diffuse distribution of NuMA similar to that seen in non-differentiated S1 cells, therefore we also assessed NuMA sensitivity to DNAse1 treatment in T4-2 cells induced to form tumor-like nodules in 3-D culture. Like in HPC2-like NuMA-CT S1 cells,

NuMA distribution was not affected by DNAse1 treatment in T4-2 cells (figure 5B). Therefore the relationship between NuMA and chromatin appears to be different between cells that have achieved acinar differentiation and cells that do not differentiate into acini.

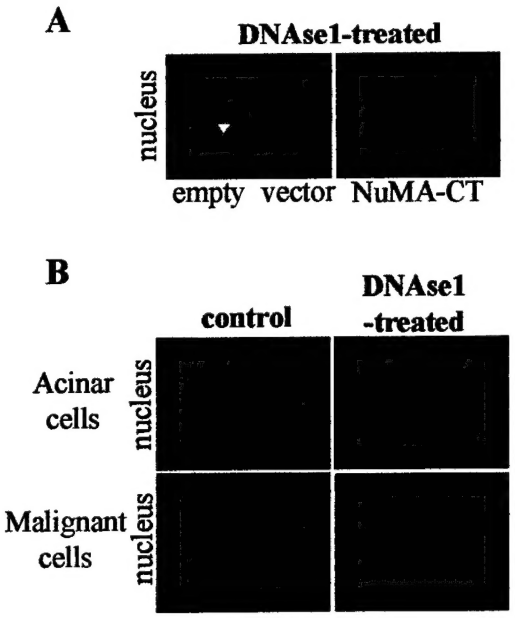

Figure 5. **The relationship between NuMA and chromatin is altered in non-differentiated HPC2-like NuMA-CT S1 cells and malignant T4-2 cells compared to acinar S1 cells. A**: Immunostaining for NuMA after DNAse1 treatment in control vector (control) and HPC2-like NuMA-CT transfected S1 cells (NuMA-CT). NuMA is mostly gone from the nucleus of control cells (except around the nucleolus [arrow]) upon DNA degradation. Whereas endogenous NuMA is hardly removed by DNA degradation in S1 cells expressing the HPC2-like NuMA-CT region (NuMA-CT). **B**: Immunostaining for NuMA after DNAse1 treatment in acinar S1 cells (upper panel) and malignant T4-2 cells (lower panel) No dramatic change is seen in malignant cells after DNAse1 treatment. One nucleus is shown per image.

Task 2 (*90% completed*): Search for NuMA binding partners.

In our previous report, we had shown that poly-his tagged HPC2-like NuMA-CT peptide used in *in vitro* affinity binding assays trapped a 65-70 kDA ligand from extracts obtained from non-neoplastic S1 cells but not from malignant T4-2 cells. However, the corresponding band on the silver stained gel was very weak. It may be very difficult to get enough material from the 3-D culture of acini to get a signal strong enough to perform mass spectrometry analysis (experiments to increase the amount of ligand trapped by the HPC2-like NuMA-CT bait are planned) and this technique brings a high background. Therefore we decided to also look for potential partners of NuMA using an approach that exploits the observation of the presence of NuMA in the soluble chromatin compartment. We prepared nuclear extracts in the presence of 0.4 M NaCl and ran these extracts on a sucrose gradient that is classically used for separation of multi-protein complexes involved in chromatin remodeling (Tanese et al., 1997). Following a 40hr centrifugation, ten 1.1 ml fractions of the gradient were TCA precipitated and run by SDS-PAGE. We also ran the nuclear pellet, which should contain non-soluble proteins, remaining after preparation of nuclear extracts. NuMA was found in a number of contiguous fractions of the gradient following centrifugation (figure 6). In contrast, lamin B was only found in the nuclear pellet. We are currently blotting the different fractions for known components of multi-protein complexes. Although NuMA seems to be found in fractions containing a well-known chromatin remodeling complex, we need to finish the analysis of the fractions with all major representatives of the different chromatin remodeling complexes before attributing NuMA to a particular multi-protein complex. Upon identification of the complexes that may include

NuMA, co-immunoprecipation experiments will be performed with the different fractions to verify the binding of NuMA to such complexes. Then we will search for the identity of the band found on silver-stained gels from the affinity binding experiments reported earlier by immunoblotting for proteins of similar molecular weight involved the formation of the multi-protein complex. This will be the last part of task 2.

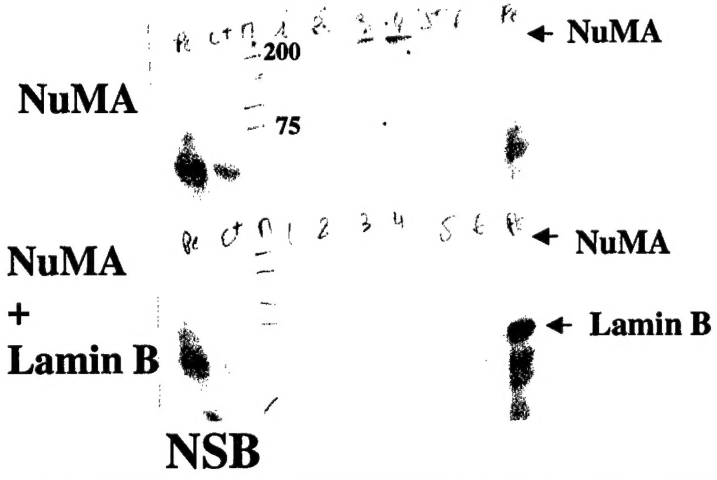

Figure 6. **NuMA is present in fractions of nuclear extracts run on sucrose gradient.** Raw preliminary data of Western blot analysis for NuMA (top image) and lamin B (performed after NuMA blotting, on the same membrane; bottom image; NuMA is still visible at the top of the film). The two first lanes were only incubated with secondary antibodies to show bands corresponding to non-specific binding (NSB). Pe= non extractable nuclear pellet, C+ = whole cell extract, M= marker (location of pre-stained bands are drawn on the film); 1.1 ml fractions 1 to 6 obtained following centrifugation are indicated; fractions 7 to 10 are not shown.

We were puzzled by the possibility that NuMA may be involved in chromatin structure. So far no enzymatic activity pertaining to chromatin remodeling have been attributed to NuMA. Our current hypothesis is that this protein may act as a receptor to organize multi-protein complexes involved in chromatin remodeling. To gain a better insight into the function of the distal part of NuMA-CT (referred to as NuMA-CTDP) where the HPC2-like domain is located, we exploited established computational techniques and tools to collate and characterize proteins with NuMA-CTDP like regions.

To date, NuMA-like proteins have been identified in *Homo sapiens* (human), *Mus musculus* (mouse), *Rattus norvegicus* (rat), *Bos taurus* (cow), and *Xenopus laevis* (frog). To identify proteins possessing NuMA-CTDP like regions, we used human NuMA residues 1915-2095, designated hNuMA-CTDP, as the protein query for TBLASTN searches against databases of genomic sequences or Expressed Sequence Tags (ESTs). hNuMA-CTDP includes a region missing in the oncogenic NuMA-RAR fusion protein (Wells et al., 1997), and the region described in task 1 believed to be involved in the control of mammary differentiation. We found related sequences showing statistically significant similarity to hNuMA-CTDP in *Sus scrofa* (pig), *Gallus gallus* (chicken), *Danio rerio (*zebrafish*)*, and *Takifugu rubripes* (puffer fish) (Figure 7). The putative pig and chicken family members were translated ESTs. For puffer fish, part of *Takifugu rubripes* genomic scaffold 307 contained short peptide matches in the -1 frame. This scaffold sequence and the Web version of GENSCAN (Burge and Karlin, 1997) were used to identify an 865 amino acid open reading frame containing the hNuMA-CTDP like region at its C-terminus. The zebrafish hNuMA-CTDP relative was identified in a similar manner using the February 2004 Ensembl assembly of the genome (http://www.ensembl.org/Danio_rerio).

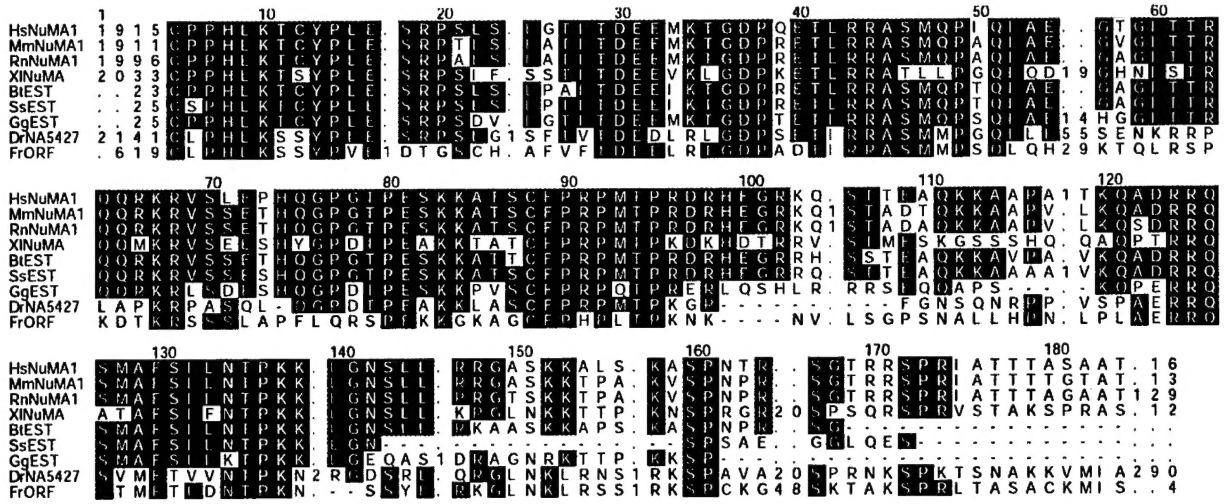

Figure 7. **HMM-generated multiple sequence alignment of the C-terminal non-coiled coil segment of NuMA family members.** The sequences shown are HsNuMA1 (*Homo sapiens* NuMA; databank code NP_006176; human), MmNuMA1 (*Mus musculus* NuMA; NP_598708; mouse), RnNuMA1 (*Rattus norvegicus* NuMA; XP_218972.2; rat), XlNuMA (*Xenopus laevis* NuMA; T30336; frog), BtEST (*Bos taurus* EST, BI680620; GI: 15633534; cow), SsEST (*Sus scrofa* EST; BX673330.1, GI: 37986836; pig), GgEST (*Gallus gallus* EST; ChEST630g3, http://chick.umist.ac.uk/ accession number: 603737612F1; chicken), DrNA547 (*Danio rerio* ENSEMBL Genscan predicted peptide; NA5427.1.260.43185; zebrafish), FrORF (*Takifugu rubripes* Genscan predicted peptide in *Fugu* scaffold 307; puffer fish). Positions conserved in at least five of the nine sequences are highlighted; the number of residues not displayed explicitly is listed.

Given the techniques and tools available currently, we found no convincing evidence for hNuMA-CT family members in three other metazoa with fully sequenced genomes (*Caenorhabditis elegans*, *Drosophila melanogaster*, and *Arabadopsis thaliana*). However, we did find what appeared to be a short match to hNuMA-CTDP in a *Ciona intestinalis* genomic scaffold. *Ciona intestinalis* is considered to be one of the earliest chordates because whilst the larval stage has a notochord, it is lost in the adult stage. These observations suggest a relatively broad phylogenetic distribution for members of the NuMA-CTDP family with convincing evidence for their presence in vertebrates (mammals: human, mouse, rat, pig; amphibians: frog; birds: chicken; and teleost fish: zebrafish and puffer fish), and a possibility that NuMA itself might be restricted to the chordate lineage.

To gain insight into the structure and thus, function of NuMA-CTDP, we sought to predict a possible three-dimensional structure for the NuMA-CTDP family. There were no matches when hNuMA-CTDP was used as the query for a National Center for Biotechnology Information (NCBI) Conserved Domain Database Search. Therefore, we estimated a hidden Markov model (HMM) from a set of diverse NuMA-CTDP sequences, hNuMA-CTDP plus its frog, chicken and teleost relatives (HsNuMA1, XlNuMA, GgEST, and FrORF, as shown in figure 1) using the Sequence Alignment and Modeling (SAM) System (http://www.soe.ucsc.edu/research/compbio/sam.html). The resulting NuMA-CTDP HMM was used to search a database of protein sequences of known three-dimensional structure provided by the Research Collaboratory for Structural Biology (RCSB, http://www.rcsb.org). The highest scoring protein of the ~55,000 sequences in the February 2004 release from the RCSB was human beta3-integrin (1m1x_B). The region of beta3-integrin identified here is

highly conserved in human beta1-and beta6-integrins. The next highest scoring protein was another membrane protein, oncoprotein receptor tyrosine kinase Her2 (1n8y). Although the scores of 1m1x and 1b8y against the NuMA-CTDP HMM were not statistically significant (E values above 1.0), the results are of biological interest because the regions of similarity correspond to largely beta extracellular domains that regulate cell-cell and cell-extracellular matrix interactions, and are involved in signaling (Borges et al., 2000; Hynes, 2002). These results suggest that the three-dimensional structure of NuMA-CTDP might be primarily beta, and that this region could be a site of intermolecular interaction(s).

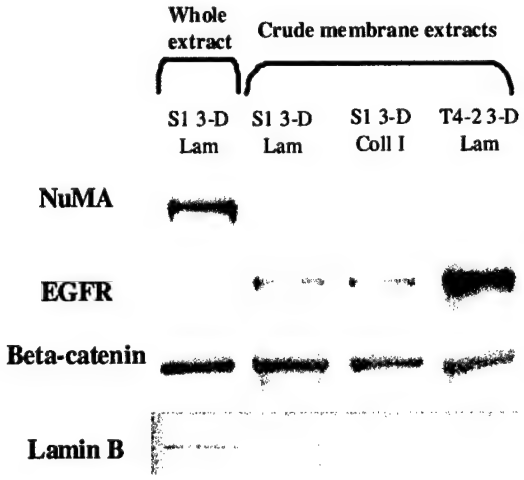

Figure 8. **NuMA is absent from the plasma membrane of HMT-3522 S1 and T4-2 cells.** Non-neoplastic S1 HMT-3522 and malignant T4-2 HMT-3522 cells were cultured for 10 days in the presence of Matrigel™ (S1 3-D Lam and T4-2 3-D Lam) or collagen I (S1 3-D Coll I). Western blots of whole cell extracts and crude membrane fractions are shown for NuMA, plasma membrane region markers epidermal growth factor receptor (EGFR) and beta-catenin, and nuclear marker lamin B.

If NuMA-CTDP is a site of interaction with other proteins, then by analogy with beta3-integrin, potential binding partners may include proteins located at the plasma membrane. More specifically this observation suggests that in addition to its known location in the nucleus, NuMA could be present at or near the cell membrane. Indeed, full length NuMA is observed in the cytoplasm of cells expressing oncogenic fusion protein NuMA-RAR (Hummel et al., 2002). Furthermore NuMA binds to protein 4.1R, a protein that participates in tethering the cytoskeleton to the plasma membrane in addition to being located at the poles of the mitotic spindle (Mattagajasingh et al., 1999). Although NuMA could be detected in the cytoplasm of non-malignant S1 HMT-3522 cells differentiated into acini, as seen on electron micrographs that we showed in our previous report, it was not located at the cell membrane and was not present in preparations of crude membrane extracts that include the plasma membrane, golgi apparatus and rough endoplasmic reticulum (Figure 8). To rule out the possibility that the absence of NuMA in membrane fractions was a characteristic of acinar differentiation, we performed the same analysis using collagen I culture of S1 cells, which induces the formation of incorrectly polarized acini, and T4-2 malignant cells that form tumor-like nodules in matrigel™ culture. NuMA was still absent from crude membrane fractions prepared under these conditions (Figure 8). The presence of NuMA in whole cell extracts was confirmed under all three conditions. The enrichment of crude membrane fractions with epidermal growth factor receptor, a marker of the plasma membrane, confirmed the successful preparation of crude membrane fractions. Thus, although NuMA appears to be located in the cytoplasm, it seems unlikely that NuMA interacts with proteins restricted to the cell surface.

TIMETABLE

Task 1: Completed

Task 2: All sub-tasks completed expect 2C (90% completed).

KEY RESEARCH ACCOMPLISHMENTS (last 18 months)

- Non-neoplastic S1 cells transfected with HPC2-like NuMA-CT do not differentiate properly (lack of complete basement membrane) and show altered chromatin organization.
- NuMA is found in the soluble chromatin compartment.
- The relationship between NuMA and chromatin in altered in cells that do not differentiate into acini, including S1 cells expressing HPC2-like NuMA-CT and malignant T4-2 cells.
- NuMA seems to be found in fractions of nuclear extracts containing multi-protein complexes.
- The distal portion of NuMA-CT that encompasses the HPC2-like domain seems restricted to chordates and is predicted to have a primarily beta strand three-dimensional structure that favors interaction with other proteins.

REPORTABLE OUTCOMES (last 18 months)

1) Patricia Abad, the research assistant working on the NuMA project obtained her Master's degree in August 2003. She is currently in the PhD program and continues to work on the NuMA project in my laboratory.
2) Patricia received an Award from the Walther Cancer Institute for her presentation on the role of NuMA in the control of mammary phenotype and chromatin organization during a regional competition involving oral presentation from graduate students.
3) Two undergraduate students, Zoltan Metlagel and Aniysha Nelpurackal, obtained their Bachelor's degree with honors due to their work on the NuMA project. They are now enrolled in graduate programs at other universities.
4) Based on the novel data suggesting a role for NuMA in chromatin structure, an RO1 application was submitted to NIH in February 2004 to explore the mechanism of NuMA function at the chromatin level and alterations of such functions upon tumor development.
5) The presence of NuMA in the chromatin compartment may explain the wide range of NuMA distributions observed with different cell phenotypes. We exploited this observation to design a novel image analysis of NuMA distribution in collaboration with David Knowles at the Lawrence Berkeley National Laboratory. The image analysis method now enables us to characterize NuMA distribution by a simple graphic representation and 'recognize' cell phenotypes based on NuMA distribution. This automated analysis will be extended to other nuclear proteins and tested for the classification of breast neoplasias on biopsies.

6) Manuscripts in which support for this project is acknowledged: a review discussing novel directions in breast cancer research partly based on the data obtained in this project was published in June 2003 [C Plachot and SA Lelièvre. "New directions in tumour biology: from basement membrane-directed polarity to DNA Methylation". Mathematical Biology and Medicine Series, "Cancer modeling and simulation", Chapman & Hall/CRC, 2003.]; a manuscript is about to be submitted on the analysis of NuMA-CT sequence [PC Abad, IS Mian, C Plachot, A Nelpurackal, C Bator-Kelly, and SA. Lelièvre. "The C-terminus of the nuclear protein NuMA: phylogenetic distribution and structure"]; two other manuscripts that deal with the function of NuMA-CT in mammary acinar differentiation are in preparation.
7) Abstracts (10 abstracts related to the project for the past 18 months, presented at local, national and international meetings) and oral presentations (six invited seminars/talks at national and international institutions or meetings during the past 18 months, see CV page 17).

CONCLUSIONS

During the past 18 months, we have demonstrated that NuMA was part of the chromatin compartment and was involved in the control of chromatin structure. The distal portion of the C-terminus of NuMA (NuMA-CTDP) seems to be critical for the relationship between NuMA and chromatin. The presence of NuMA-CTDP family members in vertebrates, and possibly the early chordate *Ciona intestinalis*, together with their likely absence in invertebrates, suggests that NuMA-CTDP may have a chordate-specific role. Given that NuMA has a role in differentiation (Lelièvre et al., 1998; Sukhai et al., 2004), one hypothesis is that NuMA-CTDP may be associated with the control of gene expression. This hypothesis is supported by the fact that expression of NuMA truncated at its C-terminus and antibodies directed against NuMA-CT induce alterations in chromatin organization (Gueth-Hallonet et al., 1998; Lelièvre et al., 1998) and by our current data showing that NuMA is part of the chromatin compartment and that NuMA-CT may be involved in the interaction between NuMA and the chromatin compartment.

The investigation of the three-dimensional structure of NuMA-CTDP is a key tool to help further decipher NuMA functions. It has been proposed that the proximal portion of NuMA-CT that contains binding sites for tubulin and LGN spanning residues 1868-1936 (Haren and Merdes 2002; Du et al., 2002), and thus is crucial for the function of NuMA during mitosis, may consist of an alpha helix structure (Haren and Merdes 2002). The potential structural similarity (beta strand) between the distal portion of NuMA-CT and the extracellular domain of beta3-integrin outlined in the present study suggests that NuMA may also possess recognition and binding functions that orchestrate nuclear and/or cytoplasmic events. Indeed the beta strand is considered to bring maximum exposure for ligand binding and may lead to intermolecular linkage (Tyndall and Fairlie, 1999; Chow et al., 2004). This secondary structure has been identified as a critical recognition element in physiological processes (Glenn and Fairlie, 2002) and is present in numerous proteins involved in signal transduction. Recently proteins bearing actin-binding domains have been proposed to play a critical role in the control of gene expression by providing a structural framework that facilitates and integrates molecular cross-talk within the nucleus (Shumaker et al., 2003). Interestingly, it was reported that NuMA possesses a calponin homology (CH) domain at its N-terminus (Novatchkova and Eisenhaber, 2002). The particular structure of NuMA

containing actin-binding (CH domain), structural (central coiled-coil domain) and organizational and signaling potential (NuMA-CTDP region) suggests that NuMA may provide a structural platform for coordinating processes in the nucleus and possibly transducing cytoplasmic signals. Thus, NuMA may become an important target in future investigation focusing on the development of differentiation strategies in breast cancer.

During the next few months, we will focus on identifying the multi-protein complexes in which NuMA is involved and based on these results, attempt to reveal the identity of the 65-70 kDA band seen on silver staining upon affinity binding experiment with HPC2-like NuMA-CT as a bait. We will also finish the manuscripts in preparation and submit them for publication.

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BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Sophie A. Lelièvre	Walther Assistant Professor of Cancer Pharmacology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Louvain, Belgium	Engineer	1984-1987	Veterinary Sciences
University of Liège, Belgium	Medical Degree	1987-1990	Veterinary Medicine
University of Paris VI, France	Master's	1990-1991	Molecular and Cell Pharmacology
University of Paris VI, France	Ph.D.	1991-1994	Molecular and Cell Pharmacology
Lawrence Berkeley Natl Lab, Berkeley, USA	postdoc	1995-1999	Mammary Cell Biology

RESEARCH AND PROFESSIONAL EXPERIENCE

1991-1995: **Veterinary Surgeon** — Pets Emergency Room, Paris District.

1991-1994: **Predoctoral Fellow** — Gustave Roussy Cancer Institute (Villejuif, France); topics: topoisomerases, anti-cancer pharmacology.

1991-1994: **Teaching assistant** — University of Paris; topics: Embryology and Histology.

1995: **Postdoctoral Fellow** — Gustave Roussy Cancer Institute (Villejuif, France), and a 3-month training in Dr Kohwi-Shigematsu's laboratory (LJCRF, La Jolla, CA); topics: resistance to topoisomerase inhibitors, metastatic phenotype, M.A.R., nuclear matrix.

1995-1997: **Postdoctoral Fellow**

1997-1999: **Postdoctoral Scientist** — Lawrence Berkeley National Laboratory; Dr Mina Bissell's laboratory (Berkeley, CA); topics: extracellular matrix-nuclear structure interrelationship, regulation of gene expression in breast morphogenesis and tumorigenesis.

1999-2000: **Research Scientist** — Cell and Molecular Biology Dept. Lawrence Berkeley National Lab., Berkeley CA; Topics: nuclear organization and gene expression, nuclear signaling.

2000-ongoing: **Assistant Professor and Walther Cancer Institute Investigator** — Basic Medical Sciences Dept., Purdue University, West Lafayette IN. Topics: nuclear organization in differentiation and cancer, nuclear signaling, nuclear structure and genomic instability.

2003-ongoing: **IU Medical School adjunct Assistant Professor** — Cancer pharmacology, Indiana University Medical School, Indianapolis, IN.

HONORS:

University of Louvain (Belgium), lifetime tuition exemption for outstanding student, 1985; **National Alexandre Joel Prize for young investigator**, 1995 (Association for Cancer Research, ARC, France); **National Prize for Fundamental Cancer Research/young investigator,** 1995 (French Society of Cancer and National Federation of Cancer Institutes, France); **Lawrence Berkeley National Laboratory Outstanding Performance Award,** 1998 (Lawrence Berkeley National Laboratory, Berkeley, CA); **Integrated Science Partnership Program Appreciation Award,** 1999 (Lawrence Berkeley National Laboratory, Berkeley, CA)

Teaching Fellowship: **University of Paris**, 1991-1994;

Research Fellowships: **French Ministry of Education and Research** (France), graduate fellowship, 1991-1994; **International Agency for Research on Cancer** (IARC-WHO), postdoctoral fellowship, 1995-1996; **Association for Cancer Research** (ARC), complementary fellowship, 1996; **Department Of Defense**/USA-Breast Cancer Research Program (Postdoctoral Training grant) 1997-1999

Collaborative Research Fellowships and Travel Awards: **French Society of Cancer Travel Fellowship,** 1995; **Journal of Cell Science Travel Fellowship,** 1997; **Philippe Foundation Travel Fellowship,** 1998; **Purdue University International Travel Award,** 2001 and 2002; **International Society of Differentiation Travel Award,** 2002.

Chair at scientific meetings: Session on "Cellular Organization, Signal Transduction and Cancer" at the "Biology and Mathematics of Cells: Physiology, Kinetics and Evolution" ESMTB meeting, Spain, 2001; co-organizer and session co-chair on "nuclear compartmentalization in differentiation and cancer" at the International Society of Differentiation meeting, France, 2002.

Guest Scientist: Lawrence Berkeley National Laboratory (2000-ongoing)

Speaker at national and international meetings:

"The solid-state signaling pathway from the extracellular matrix to the nuclear matrix: the critical role of 3D architecture at the cellular level", High resolution X-ray CMT Workshop (LBNL, Berkeley, CA), August 1996; ***"Internal cell architecture-A new look"***, Advanced Light Source Users Meeting (LBNL, Berkeley, CA), October 1997; ***"Global rearrangement of nuclear matrix-associated proteins when human mammary epithelial cells are cultured in 3-D: an analysis using confocal-, electron-, and soft x-ray microscopy"***, Keystone Symposium on Nuclear Matrix (Copper Mountain, CO; junior investigators workshop), April 1998; ***"Nuclear structure, cell proliferation, and tissue morphogenesis"***, American Society for Cell Biology Meeting (San Francisco, CA), December 1998; ***"Tissue architecture and gene expression: study of tissue matrix in three-dimensional models of cell culture"*** and ***"The non-chromatin structure of the nucleus or nuclear matrix: study of its interaction with the chromatin structure and its role in the regulation of gene expression"***, Biomathematics Summer School (Termoli, Italy), Mathematics in Cell Physiology and Proliferation, June 1999; ***"Nuclear-directed signaling in mammary gland acini"***, Gordon Conference on Biological Structure and Gene Expression (Meriden, NH; short talk), August 1999; ***"Nuclear organization in normal and malignant breast: NuMA is a marker of cell phenotype and a regulator of differentiation"***, Era of Hope DOD Breast Cancer Research Meeting (Atlanta, GA; platform talk), June 2000; ***"Cell cycle regulation in higher order cell assemblies: the role of three-dimensional tissue architecture,"*** Third International Congress of Nonlinear Analysts (Catania, Sicily) July 2000; ***"Signal transduction and feedback signaling"***, ***"Cellular transformation and genomic instability"***, ***"Tumor progression: How in vitro models may help understand in vivo situations"***, ESMTB School, Biology and Mathematics of Cells: Physiology, Kinetics and Evolution, (Siguenza, Spain) June 2001. ***"NuMA functionally links cell adhesion and nuclear structure to regulate cell survival in breast"***, 2nd International Conference on Tumor Microenvironment: Progression, Therapy and Prevention, (Baden, Austria), June 2002; ***"Multiple facets of nuclear structural proteins: The Role of NuMA is the regulation of breast epithelial phenotypes"***, 12th Internatioanl Conference of the International Society of Differentiation, (Lyon, France), September 2002; ***"link between compartmentalization and functions of nuclear proteins in phenotypically normal and neoplastic tissues"***, FASEB Summer Research Conference on "Nuclear Structure and Cancer", (Saxtons River, VT), June 2003; ***"Proteomics with a twist-Early detection of breast cancer by looking beyond protein expression"***, The Amelia Project-Giving Wings to Research, (Indianapolis, IN), February 2004; ***"Tissue structure and gene expression control"***, Research in Cell Therapy Conference, Session on proliferation and differentiation in normal and pathological cells, (St Louis Hospital, Paris, France), March 2004.

Selected Seminars (since 2000):

"The organization of the cell nucleus in breast differentiation and tumorigenesis. A source for the development of novel anticancer strategies" Research Institute of MolecularPathology, Vienna Biocenter (Boehringer-Ingelheim,Vienna, Austria), April 2000; ***"Structure, Instability and Plasticity in Cancer"*** University of Mexico, Mexico City, September 2001; ***"Subcompartmentalization of Nuclear Proteins in Differentiation and Cancer: Multi-faceted NuMA Regulates Breast Epithelial Cell Behavior."*** IUPUI, Indianapolis, IN, November 2001. ***"Chromatin structure and breast differentiation: The role of the supramolecular organization of nuclear proteins"*** Boston University, Boston, MA, April 2003; ***"Architectural proteomics –When proteins become stars"***, Lawrence Berkeley National Laboratory, Life Sciences Division, (Berkeley, CA), February 2004.

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Knowles, D Sudar, CM Bator Kelly, MJ Bissell and **SA Lelièvre**. "Novel image analysis links the sub-

nuclear distribution of NuMA with alterations in mammary cell phenotype". (In preparation).
PC Abad, J Lewis, S Mian, and **SA Lelièvre**. "NuMA regulates chromatin structure in interphase". (In preparation).